Simultaneous Exfoliation and Functionalization of 2H-MoS_2 by Thiolated Surfactants: Applications in Enhanced Antibacterial Activity

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Contents

Ligand Synthesis

Thiol ligands are synthesized according to previous report [1,2] and non-thiol ligands are prepared as follows

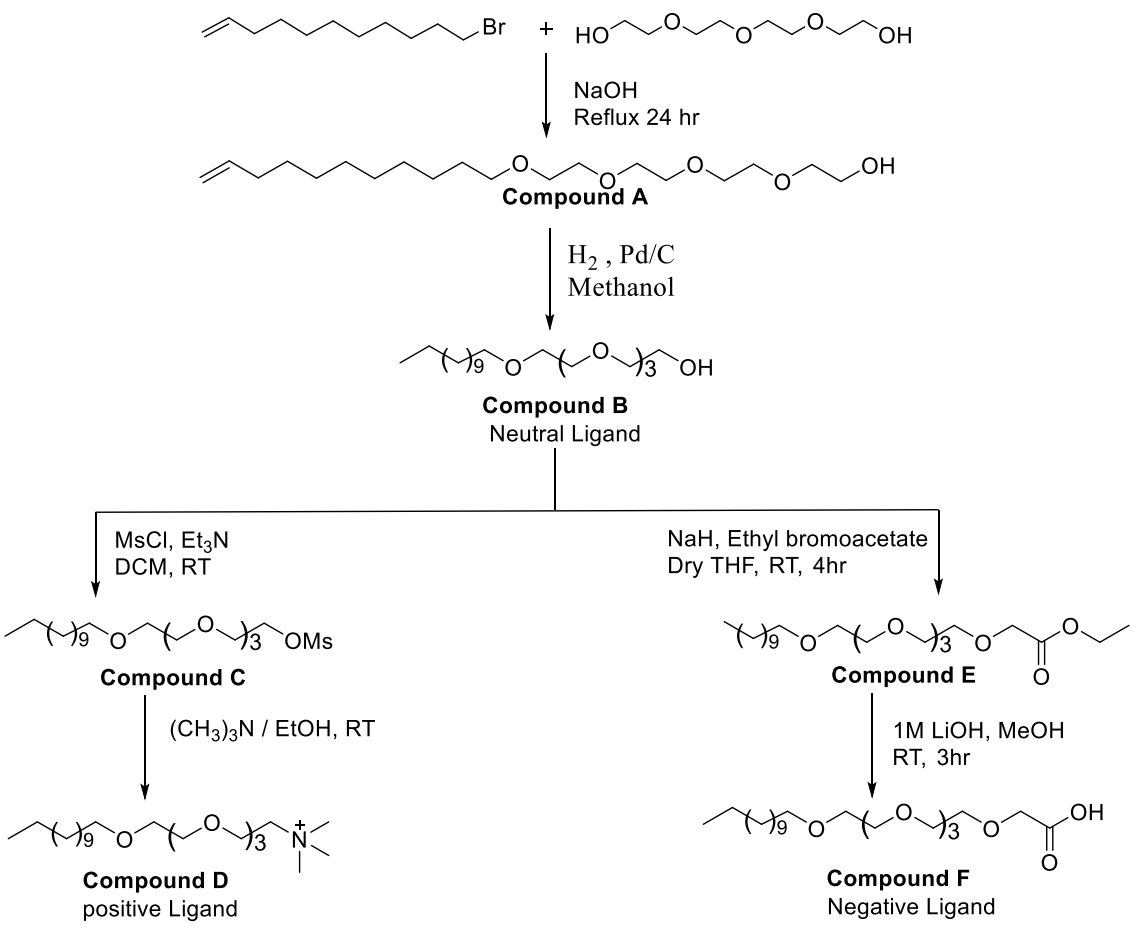

Figure S1. Scheme for the synthesis of various non-thiol ligand for exfoliation of TMDs.

All the steps in the non-thiol ligands are like thiol ligand synthesis as in the previous report, except the reduction of double bond instead of introduction of thiol by AIBN reaction. In short procedure for non-thiol ligand synthesis are

1. Synthesis of Compound A

A mixture of tetraethylene glycol (106.5 mmol) and sodium hydroxide (21.4 mmol) was stirred about 30 min at 100℃ under argon atmosphere. Then 11-bromoundec-1-ene (21.4 mmol) was added slowly. The reaction mixture was cooled to room temperature after 24 h. The crude product was extracted from reaction mixture using hexane repeatedly for six times. Combined hexane portions concentrated in rotary evaporator, gives yellow oil containing a mixture of

mono-alkylated and di-alkylated product. The mono-alkylated product was purified by column chromatography with a yield of 76%.

2. Synthesis of Compound B (Neutral non-thiol ligand)

Compound A (8.6mmol) dissolved in methanol with 10 wt% of Pd/C taken in a round bottom covered with septum and in the presence H_2 gas bubbled through the solution with syringe needle. The reaction carried-out for 12 h, after completion of reaction the solvent is evaporated by rotary evaporator. The crude reaction mixture re-dissolved in ethyl acetate and filtered Pd/C by column by using silica gel (100-200 mesh). The purified product was characterized by ^{1}H-NMR with yield of 98%.

^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.642-3.571 (m, 16H, -O-CH_2-CH_2-O-), 3.465-3.430 (t, 2H, -CH_2-$\underline{CH_2}$-O-), 1.595-1.560 (m, 2H, -$\underline{CH_2}$-CH_2-O-), 1.258 (s, 16H, -CH_2), 0.896-0.862 (t, 3H, -CH_3).

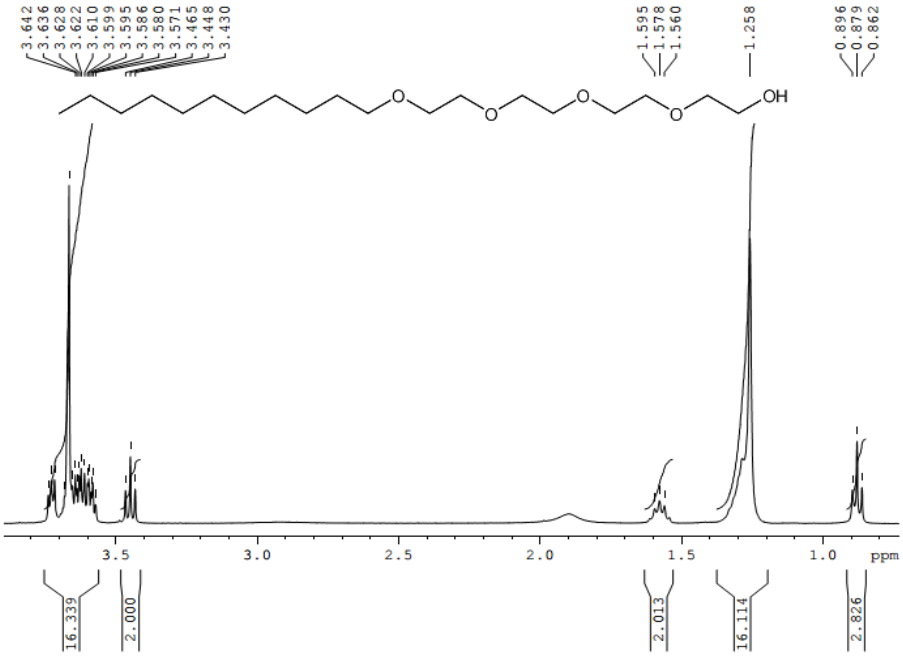

Figure S2. ^{1}H-NMR spectra of Compound B (Neutral non-thiol ligand)

3. Synthesis of Compound C

Compound B (3.2 mmol) dissolved in 20 mL of DCM taken in an RB which kept in 0℃. The triethylamine (4.8 mmol) was added to the reaction mixture at 0℃ and stirred for 15 minutes. Then methane sulfonyl chloride (4.8mmol) was added slowly to the reaction mixture and allowed stirred at room temperature overnight. After completion of the reaction, evaporated the DCM, and dissolved the crude mixture in ethyl acetate followed by washing twice with dilute HCl (0.1M) and saturated sodium bicarbonate solution. The organic layer was dried using anhydrous sodium sulfate, concentrated in vacuum and purified by column chromatography. The product was confirmed by ^{1}H-NMR with a yield of 85%.

4. Synthesis of Compound D (Positive non-thiol ligand synthesis)

Dissolved Compound C (3 mmol) in 10 mL of EtOH, then added 6 mmol of trimethylamine (33% in EtOH). The reaction was allowed stir at room temperature under argon atmosphere until the disappearance of starting material, monitored by TLC. Trimethylamine was added, if required at regular interval. The trituration method is used to purify the product in hexane: ether (1:1) and kept in the refrigerator for overnight. The precipitated product was collected and characterized by ^{1}H-NMR with a yield of 92%.

^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.830 (Br, 2H, O-CH_2-$\underline{CH_2}$-N+), 3.818-3.672 (t, 2H, O-$\underline{CH_2}$-CH_2-N+), 3.666-3.560 (m, 12H, -O-CH_2-CH_2-O-), 3.454-3.420 (t, 2H, -CH_2-$\underline{CH_2}$-O-), 3.361 (s, 9H, -N+ $(CH_3)_3$), 2.741 (s,3H, -OMs), 1.584-1.550 (m, 2H, -$\underline{CH_2}$-CH_2-O-), 1.259 (s, 16H, -CH_2), 0.896-0.862 (t, 3H,-CH_3).

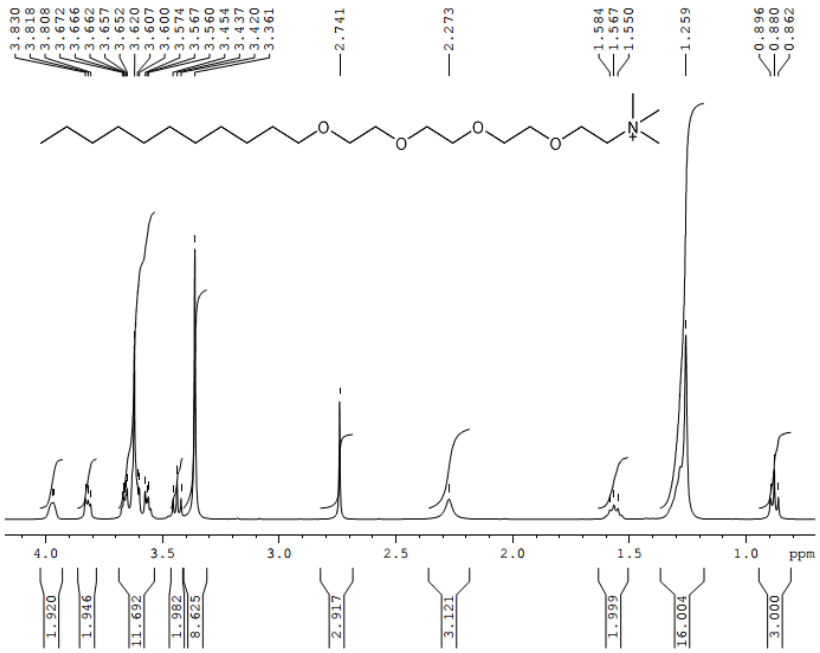

Figure S3. ^{1}H-NMR spectra of Compound D (Positive non-thiol ligand)

5. Synthesis of Compound E

Compound B (3 mmol) was taken in RB kept in argon atmosphere and dissolved in dry THF. Separately sodium hydride (4.5 mmol) was taken in another RB and kept at 0℃ under argon atmosphere, then added 5 mL of dry THF. The compound B solution was added to the RB containing sodium hydride in THF. Then ethyl bromoacetate (4.5 mmol) was added after 10 min slowly to the reaction mixture in cold condition under argon atmosphere. The reaction mixture allowed stir at room temperature for 4 h. The reaction was quenched by adding few drops of water and the crude mixture was concentrated using rotatory evaporator. Then the crude mixture was dissolved in ethyl acetate and washed twice with brine solution. The concentrated organic layer was subjected to column chromatography for purification. The purified product was confirmed by ^{1}H-NMR with a yield of 68%.

6. Synthesis of Compound F (Negative non-thiol ligand)

Compound E (2.5 mmol) was dissolved in 10 mL of MeOH, then added 2 mL of 1M lithium hydroxide and stirred at room temperature for 3 h. After completion of reaction, the solution was allowed cool to 0℃. And acidified to a pH 2 by addition of 1M HCl. Then evaporated THF, re-dissolved in ethyl acetate and washed with brine for three times. The concentrated organic layer

was purified by column chromatography and the purified product was confirmed by ^{1}H-NMR with yield of 98%.

^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.752 (s, 2H,-O-CH_2-CO-), 3.745-3.578 (m, 16H, -O-CH_2-CH_2-O-), 3.472-3.438 (t, 2H, -CH_2-$\underline{CH_2}$-O-), 1.592-1.540 (m, 2H, -$\underline{CH_2}$-CH_2-O-), 1.258 (s, 16H, -CH_2), 0.896-0.862 (t, 3H,-CH_3).

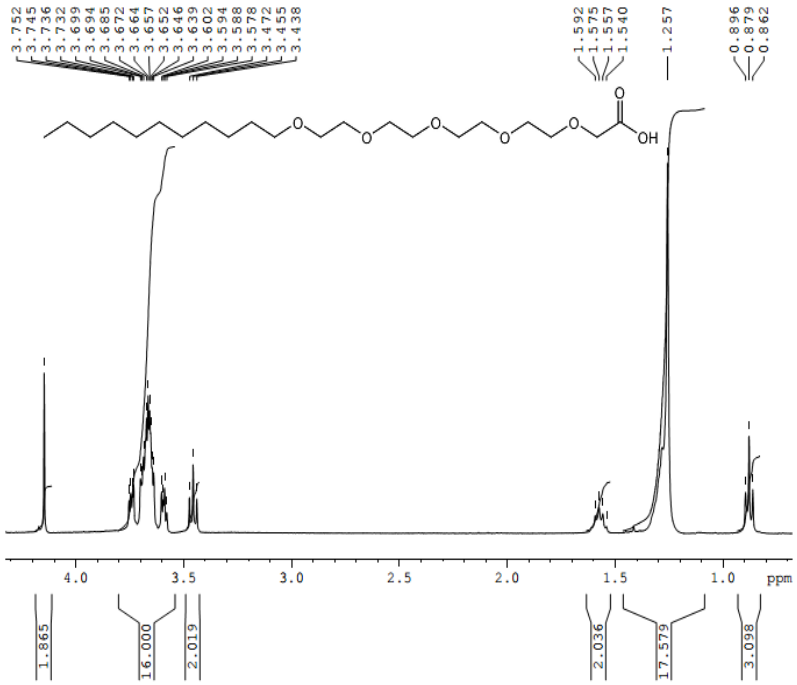

Figure S4. ^{1}H-NMR spectra of Compound F (Negative non-thiol ligand).

7. Optimization of ligand concentration for exfoliation of TMD

In a typical sonochemical exfoliation experiment, 5 mg of bulk MoS_2 was added to a 15 mL water solution containing different equivalence (0.5, 1, 1.5 & 2) of **ligand 1** with respect to molecular weight of MoS_2. This mixture was sonicated by probe sonicator (amplitude-30%, frequency- 20 kHz, Watt-500 W,) over a period of 80 min (Time-10 min, Pulse- 55sec on, 5 sec off) and the progress of exfoliation was monitored by UV-vis spectroscopy by plugging 500 µL of sample at different time interval and centrifuged at 10000 rpm throw the supernatant. Further precipitate re-dispersed in water and centrifuged at 2000 rpm, collected the supernatant made volume to 1 mL and measured absorbance by UV-vis spectrometer.

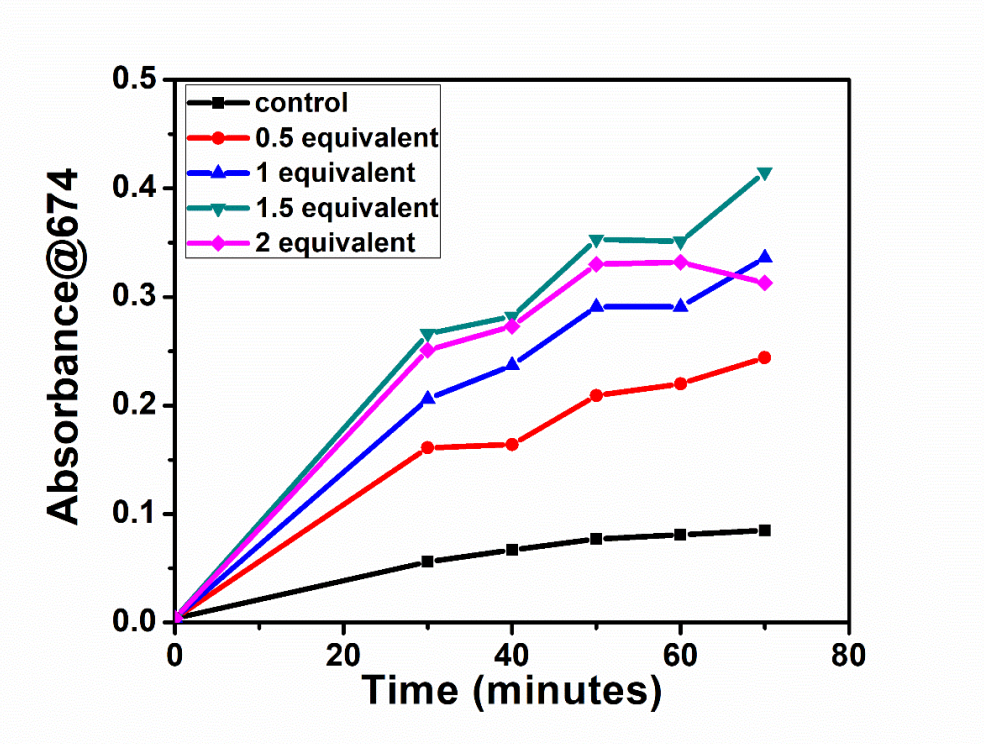

Figure S5. a) Temporal evolution of absorbance at 674 nm of MoS_2 with **ligand 1**, which reflects exfoliation rate of MoS_2 with different concentration of **ligand 1**.

8. Effective dispersion of bulk MoS_2 in the presence of thiol ligand in water

a)

b)

c)

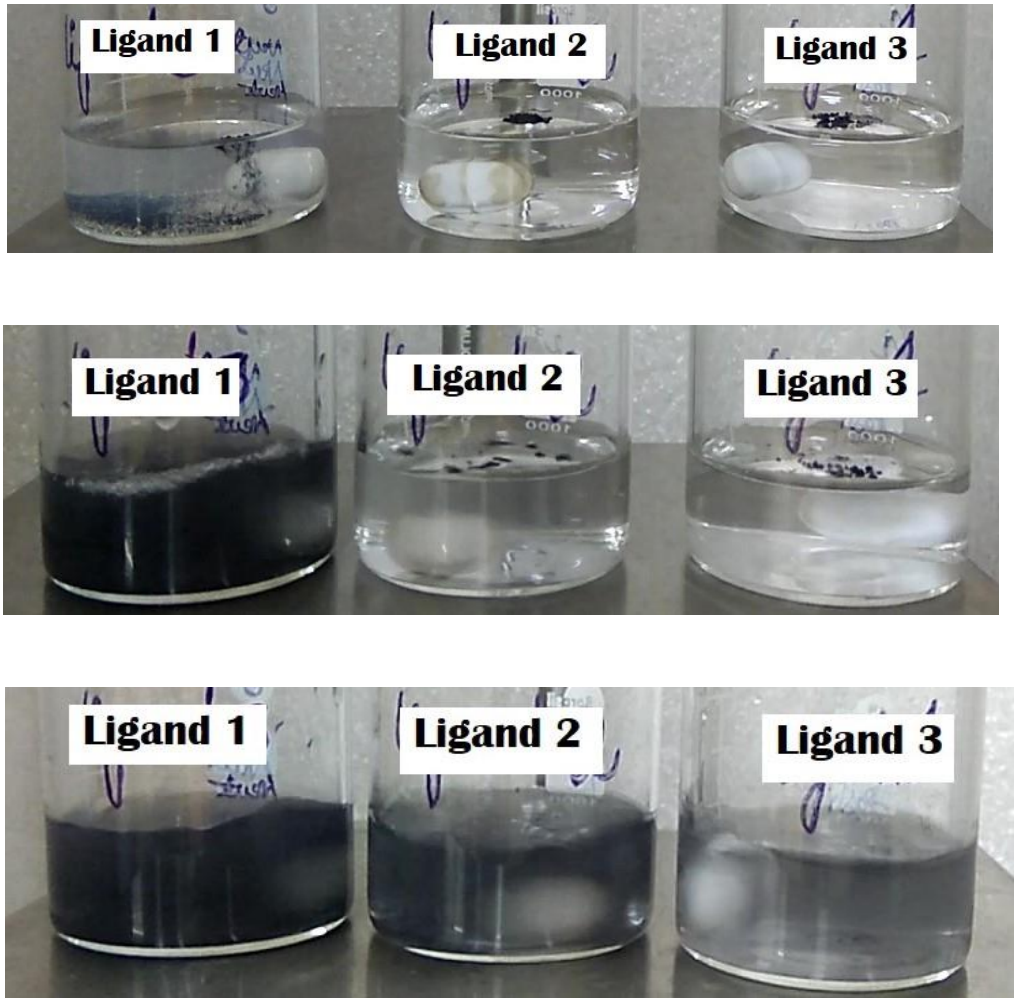

Figure S6. Dispersion ability of bulk MoS_2 with different thiol ligand in water. a) After adding bulk MoS_2, ligand containing aqueous solution, only **ligand 1** induces breakage of bulk MoS_2, whereas **ligand 2** and **3** results in floating. b) Stirring for 30 sec **ligand 1** completely disperse the bulk MoS_2 in the solution. c) Continuous stirring for one-minute **ligand 1** results faster and complete dispersion of bulk MoS_2, whereas **ligand 2** and **ligand 3** are slower and less dispersion of bulk MoS_2.

9. NMR of functionalized MoS_2

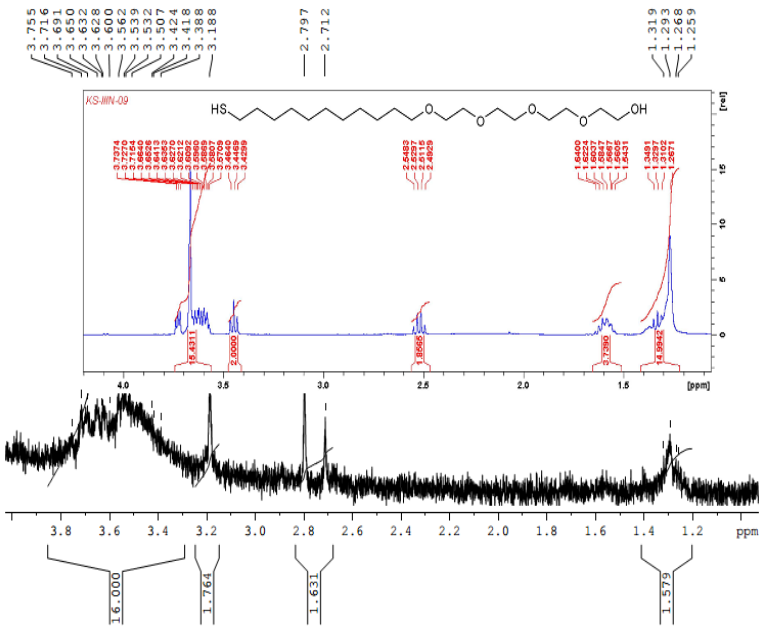

Figure S7. ^{1}H-NMR of **ligand 1** functionalized MoS_2 in D_2O.

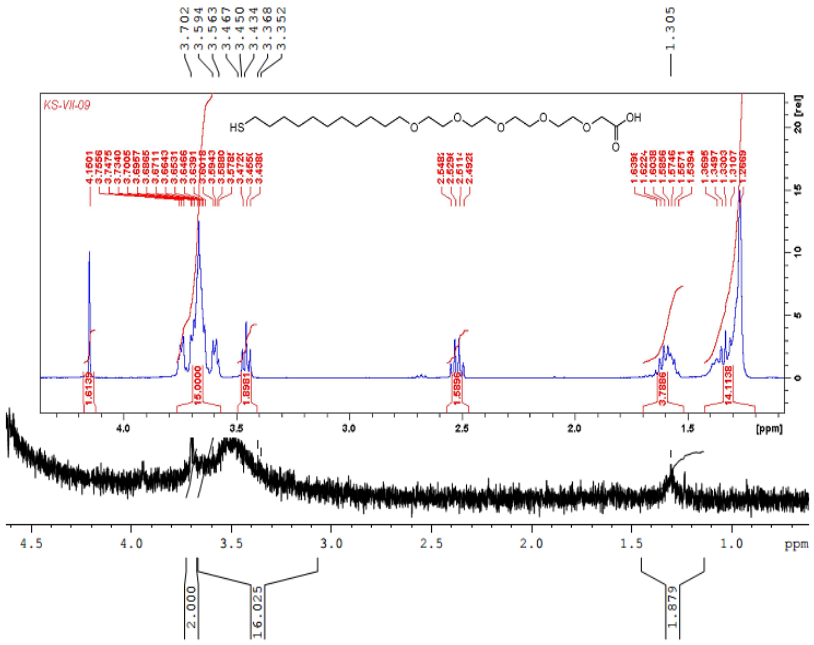

Figure S8. ^{1}H-NMR of **ligand 2** functionalized MoS_2 in D_2O

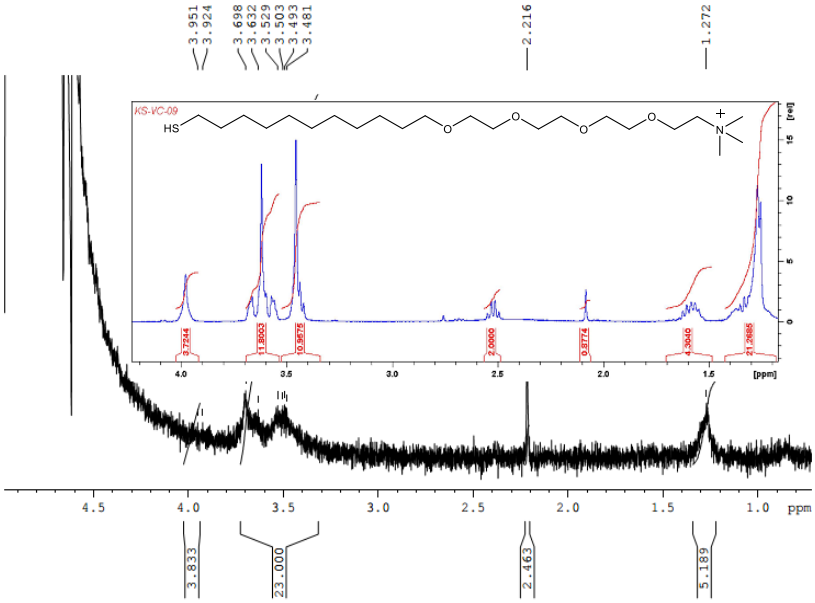

Figure S9. ^{1}H-NMR of **ligand 3** functionalized MoS_2 in D_2O

10. Thermo gravimetric analysis (TGA) of ligand 3 functionalized MoS_2

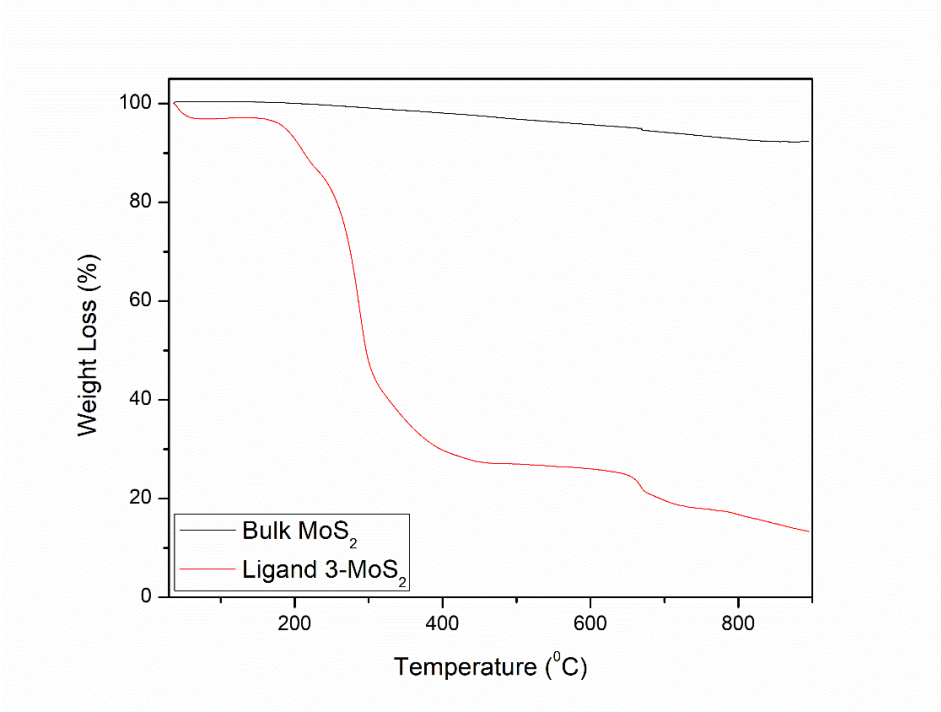

Figure S10. TGA spectra of **ligand 3** functionalized MoS_2.

11. Large area HRTEM of ligand 6 induced exfoliated MoS_2.

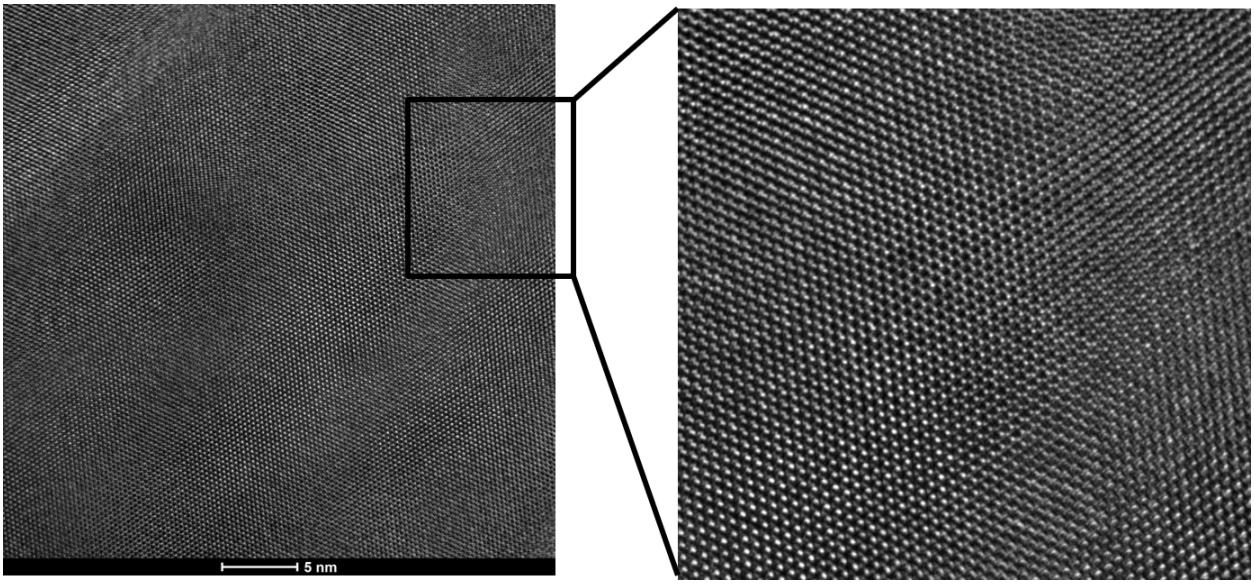

Figure S11. Large area HRTEM of **ligand 6** -MoS_2 which indicates no tears and structural distortion in the basal plane.

12. Photoluminescence of functionalized MoS_2

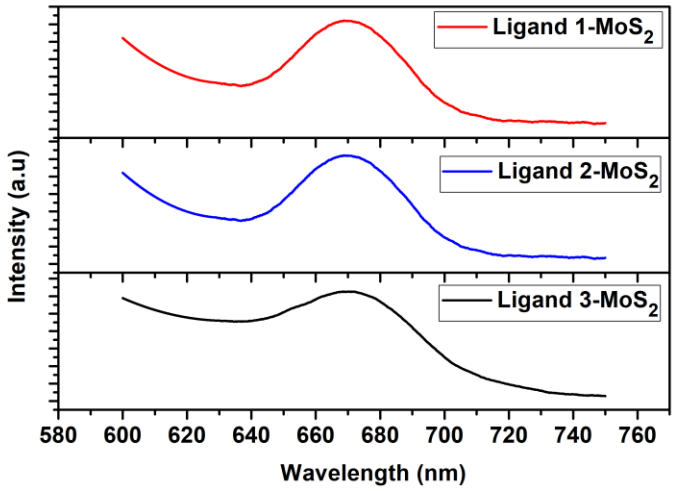

Figure S12. Photoluminescence of exfoliated MoS_2 by different thiol ligands measured using Lab Ram HR with excitation wavelength 532 nm.

13. AFM of exfoliated MoS_2 with various ligands

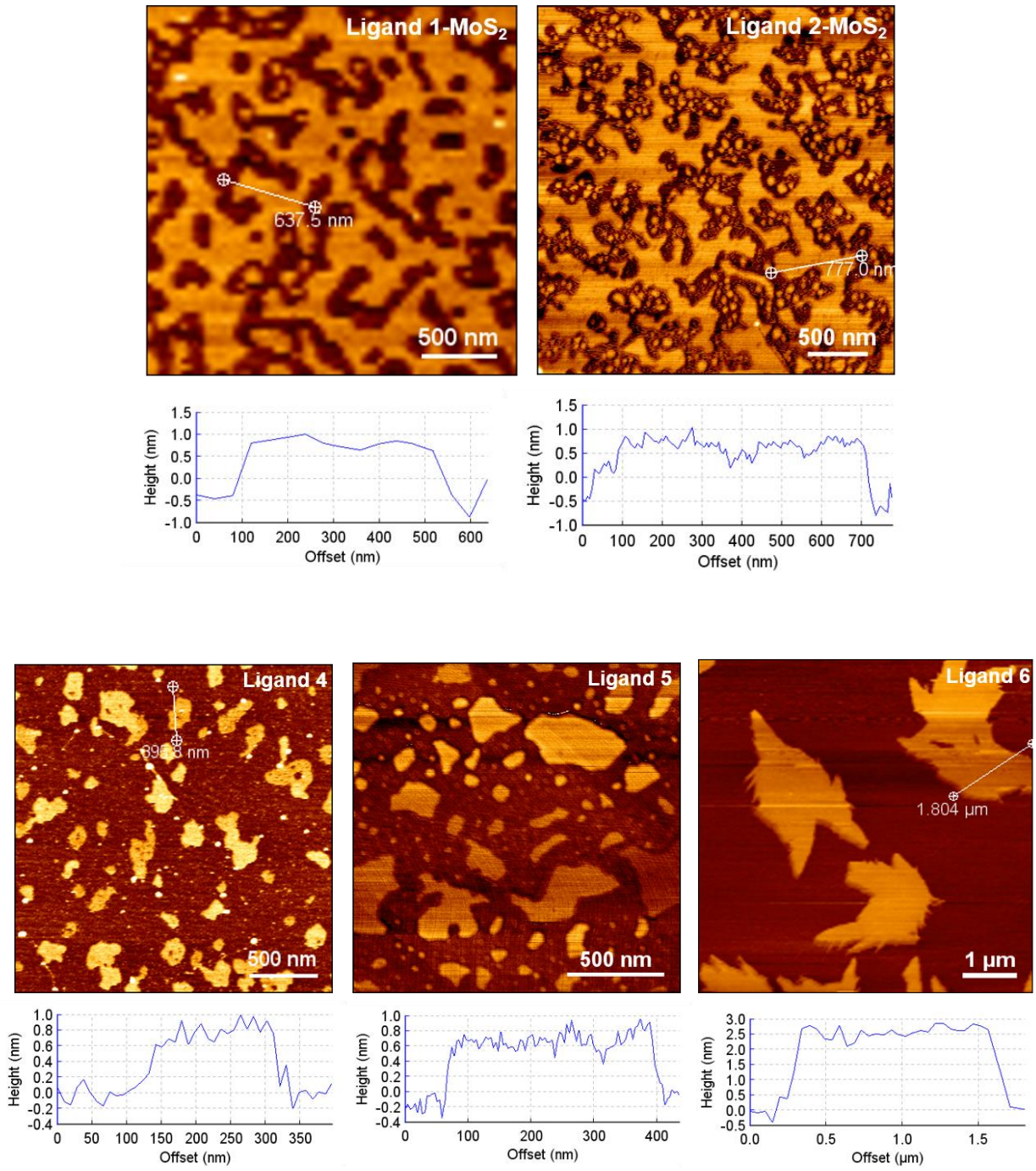

Figure S13. AFM of exfoliated MoS_2 with different ligands and the corresponding height profile diagram.

14. TEM of exfoliated MoS_2 with different thiol ligands

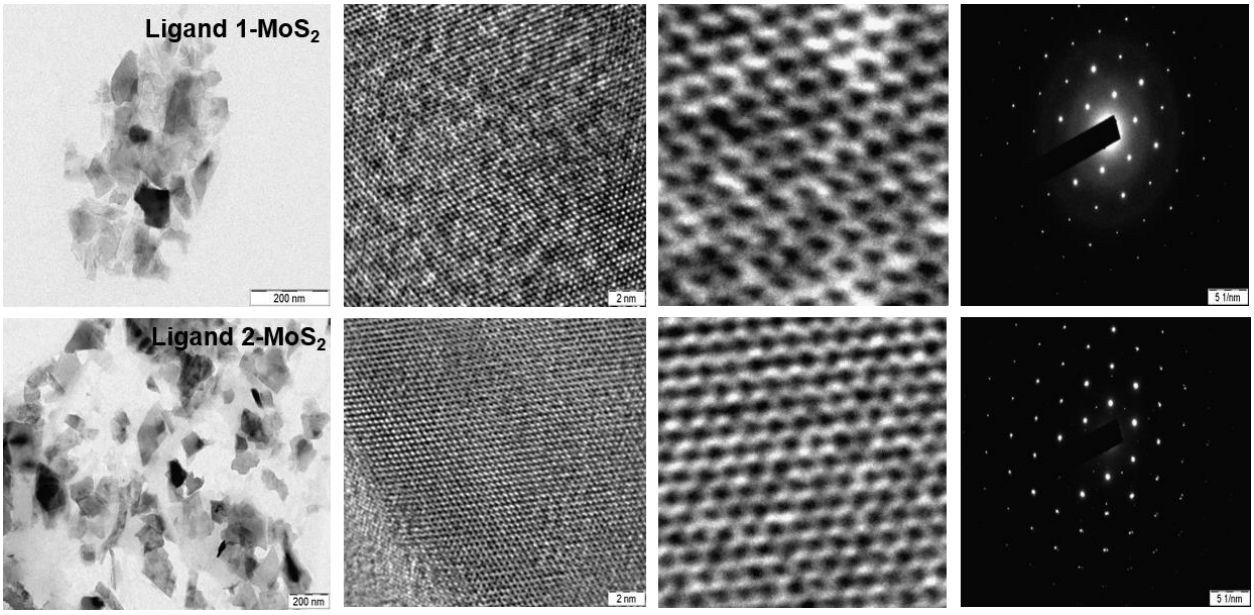

Figure S14. TEM of exfoliated MoS_2 with different ligands and the corresponding HRTEM and SAED pattern.

15. AFM of exfoliated TMDs with ligand 4

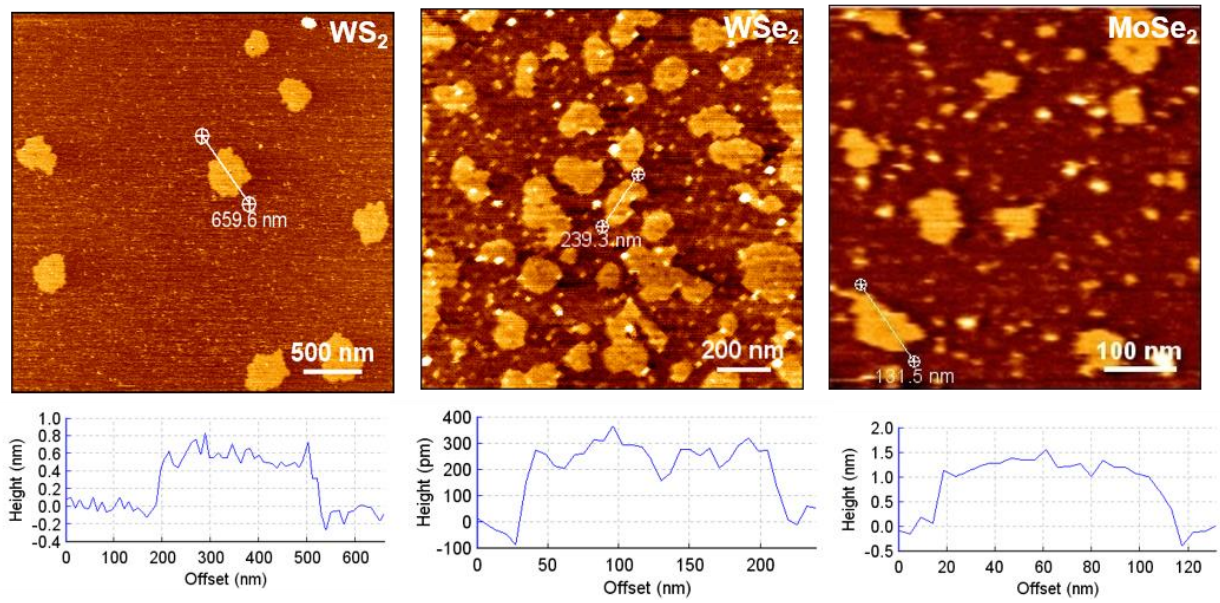

Figure S15. AFM of exfoliated TMDs using **ligand 4** and the corresponding height profile diagram.

16. TEM of exfoliated TMDs with ligand 4

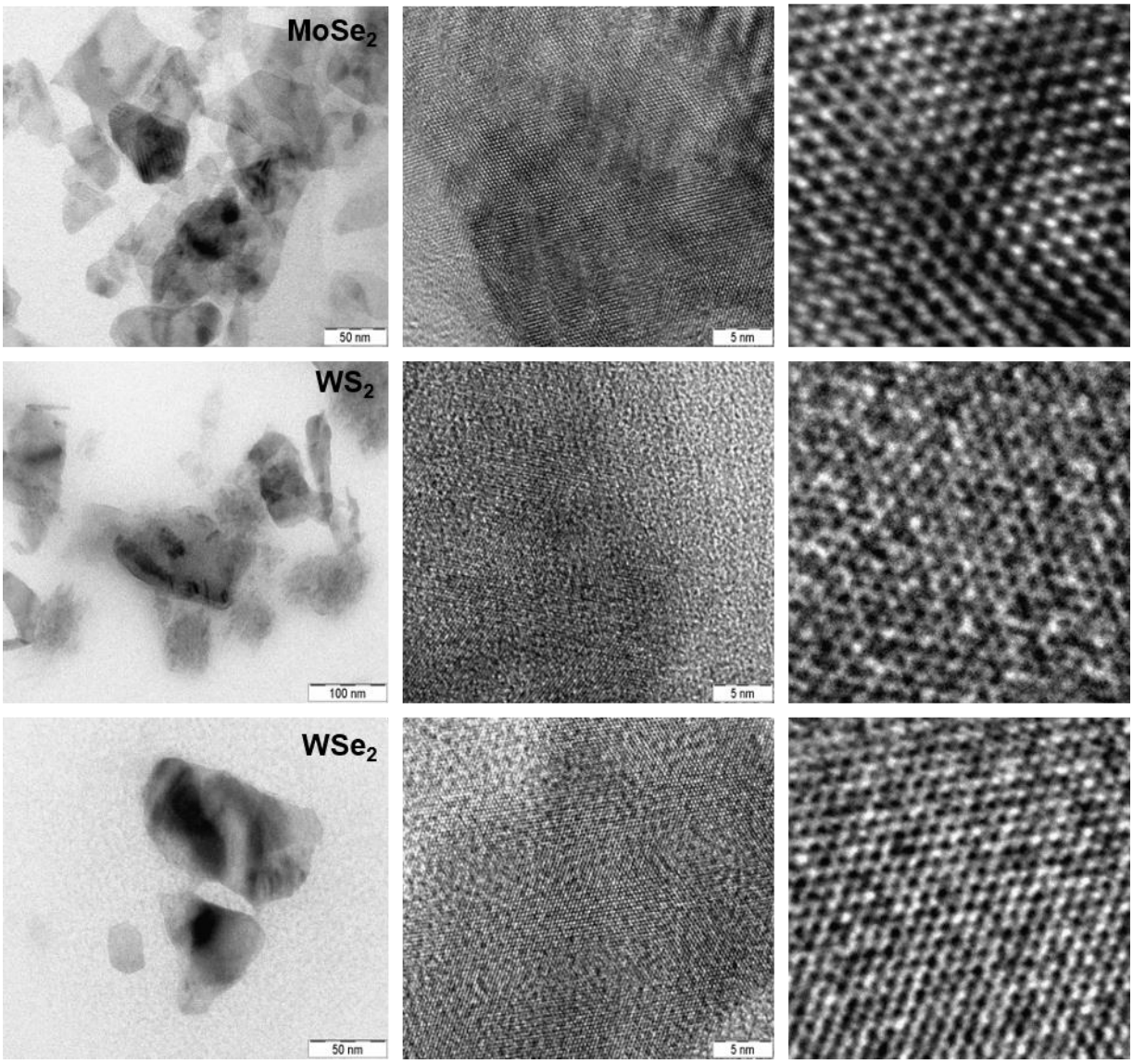

Figure S16. TEM of exfoliated TMDs using **ligand 4** and the corresponding HRTEM.

17. Minimum inhibitory concentration and minimum bacteriocidal concentration (MIC and MBC) for different Positive MoS_2

The antibacterial activity of the functionalized MoS_2, tested using Methicillin Resistant *Staphylococcus aureus* (MRSA, USA300) and *Pseudomonas aeruginosa*. Revived the freeze-dried bacterial species on nutrient agar plates. Primary culture was prepared by few colonies of the bacteria from agar plate were cultured in Luria broth media (LB, HiMedia – 20 g/L) overnight for 10 -12 h. For secondary culture 50 µL of primary culture was sub-cultured in 5 mL of fresh LB until it reaches the mid-log phase (A_{600nm}~0.3). The optical density of the seeding bacteria was adjusted to A_{600nm} = 0.01 (10^6 to 10^7 bacteria/ mL) and used for the experiments.

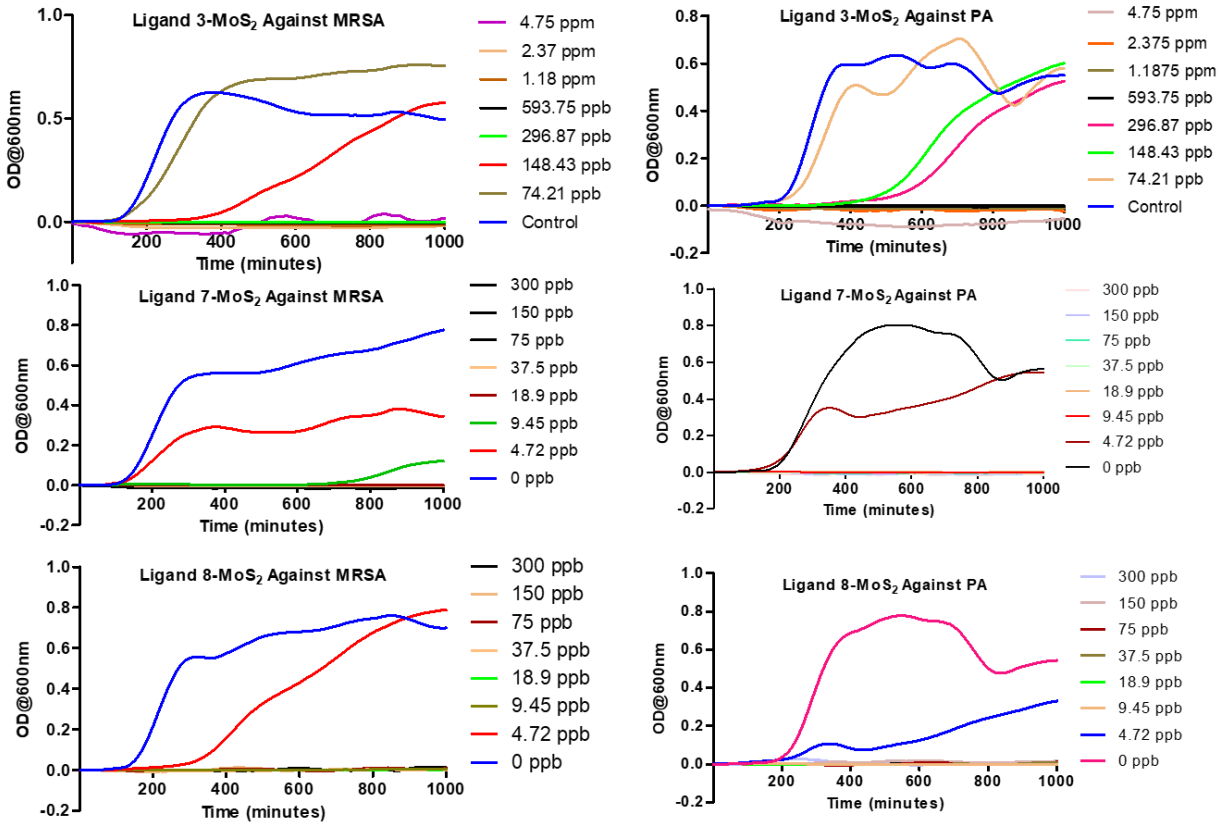

Figure S17. Bacterial Growth curve of two different bacterial strains (MRSA & *P. aeruginosa*) after treating with different positive MoS_2.

The microbroth dilution method in 96-well plates was used to estimate minimum inhibitory concentrations (MIC) of functionalized MoS_2. Phosphate Saline Buffer (PBS) was used to prepare two-fold serial dilution of the working solution concentrations. Bacterial suspension 100

μL with A_{600nm} = 0.01 added 100 μL of the working solutions prepared in 96 -plate. The bacterial growth curves were monitored using micro-plate reader (Eppendorf AF2200) equipped with a shaker and thermostat set to 37℃ over a period of 16 h. In a real time, kinetic cycle optical density at 600 nm taken at 10 min intervals followed by orbital shaking at 100 rpm. The minimum concentration at which there was no rise or 95% decline in the growth curves was designated as minimum inhibitory concentration (MIC).

For minimum bactericidal concentration (MBC) determination, after growth curve measurement, the 96 well plates were incubated at 37℃ for 4 h and then bacterial solution from the treated wells were taken and streaked on a nutrient agar plate. The minimum concentration at which no bacterial growth was observed has been designated as MBC.

18. Zeta potential of ligand 7 and ligand 8 functionalized MoS_2

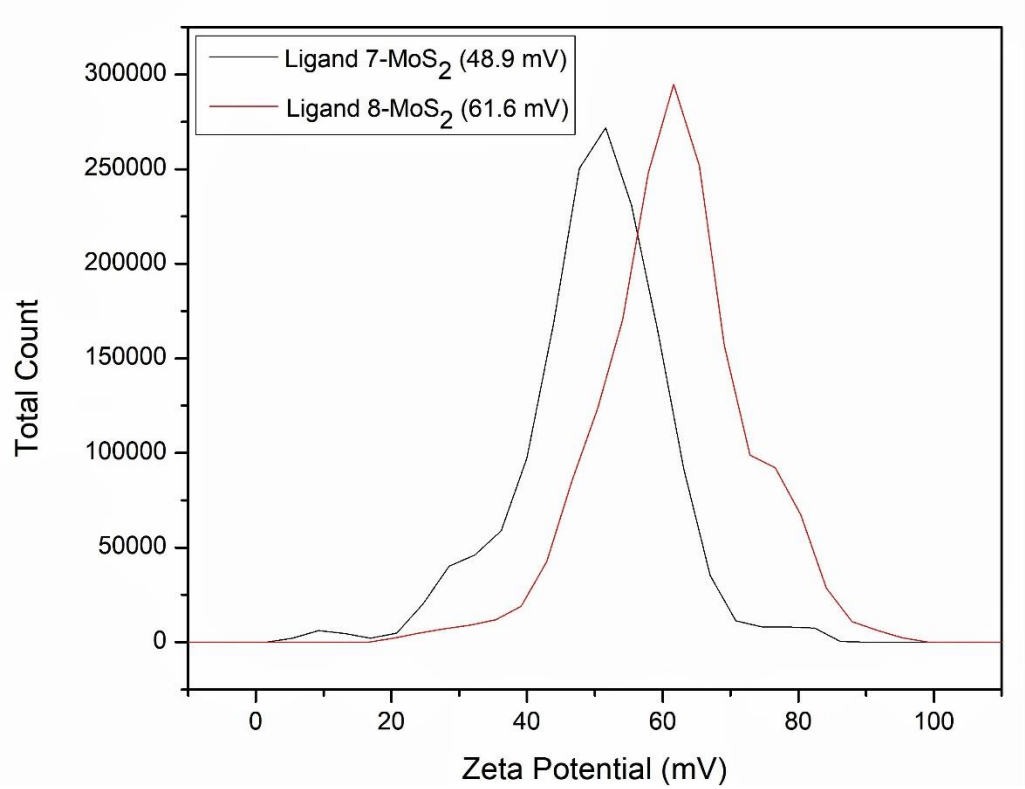

Figure S18. Zeta potential of the functionalized MoS_2 with **ligand 7** and **ligand 8** has been measured in 5 mM sodium phosphate buffer, pH 7.4.

19. Growth kinetics and doubling time MRSA and *P. aeruginosa*

The Growth Kinetics (μ)and doubling time (t_d) calculated using following equations.

$$\mu = \frac{2.303(\log OD_2 - \log OD_1)}{(t_2 - t_1)}$$

$$t_d = \frac{\ln(2)}{\mu}$$

Where optical density 1 (OD_1) is the starting point of the exponential phase at time (t_1) and the optical density 2 (OD_2) is end of exponential phase at time (t_2).

Table S1. Growth kinetics and doubling time of MRSA

MRSA								
Conc (ppb)	300	150	75	37.5	18.75	9.45	4.72	0
C8-MoS_2 μ (h^{-1})	NG	NG	NG	NG	NG	NG	0.288	1.11
C8-MoS_2 t_d (h)	NA	NA	NA	NA	NA	NA	2.4	0.6
C6-MoS_2 μ (h^{-1})	NG	NG	NG	NG	NG	NG	0.251	1.05
C6-MoS_2 t_d (h)	NA	NA	NA	NA	NA	NA	2.75	0.6
Conc	4.75	2.375	1.187	0.593	0.296	0.148	0.074	0
C1-MoS_2 μ (h^{-1})	NG	NG	NG	NG	NG	0.281	0.66	0.973
C1-MoS_2 t_d (h)	NA	NA	NA	NA	NA	2.46	1.04	0.712

No Growth-NG, Not applicable-NA

Table S2. Growth kinetics and doubling time of *P. aeruginosa*

P. aeruginosa								
Conc (ppb)	300	150	75	37.5	18.75	9.45	4.72	0
C8-MoS_2 μ (h^{-1})	NG	NG	NG	NG	NG	NG	0.176	0.942
C8-MoS_2 t_d (h)	NA	NA	NA	NA	NA	NA	3.91	0.73
C6-MoS_2 μ (h^{-1})	NG	NG	NG	NG	NG	NG	0.345	0.63
C6-MoS_2 t_d (h)	NA	NA	NA	NA	NA	NA	2	1.08
Conc	4.75	2.375	1.187	0.593	0.296	0.148	0.074	0
C1-MoS_2 μ (h^{-1})	NG	NG	NG	NG	0.339	0.418	0.991	1.147
C1-MoS_2 t_d (h)	NA	NA	NA	NA	2.04	1.65	0.699	0.603

No Growth-NG, Not applicable-NA

20. Effect of ligand on antibacterial activity

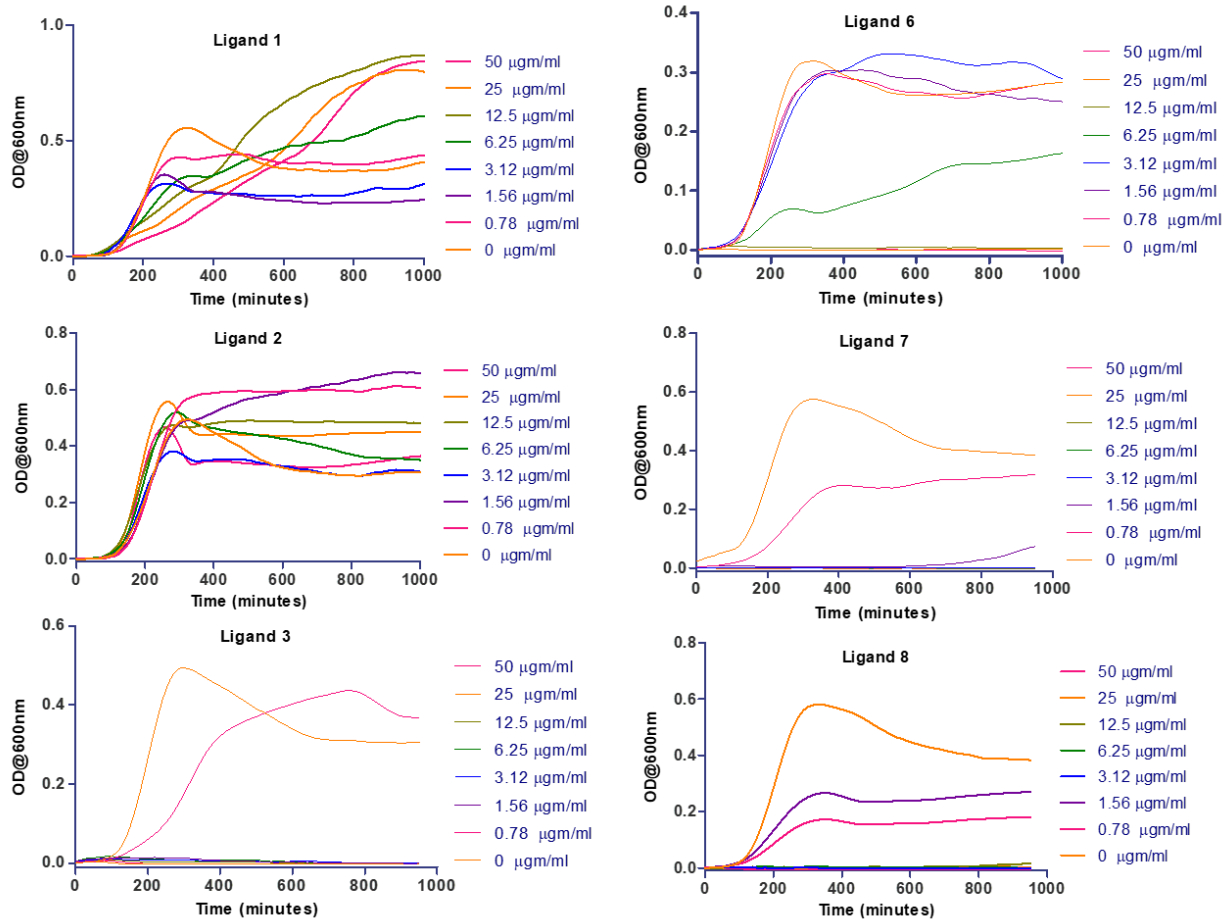

Figure S19. Effect free ligands all thiol (**ligand** 1, 2, 3, 7 and 8) and non-thiol ligand (**ligand** 4) on growth of MRSA.

Table S3. MIC Value of Free ligands.

Ligands	MIC	Ligands	MIC
Ligand 1	No Inhibition	Ligand 6	12.5 µg/ml
Ligand 2	No Inhibition	Ligand 7	3.12 µg/ml
Ligand 3	1.56 µg/ml	Ligand 8	3.12 µg/ml

21. Comparison 2H-functionalized MoS_2 efficacy with other nanomaterial and small molecule-based antibiotics.

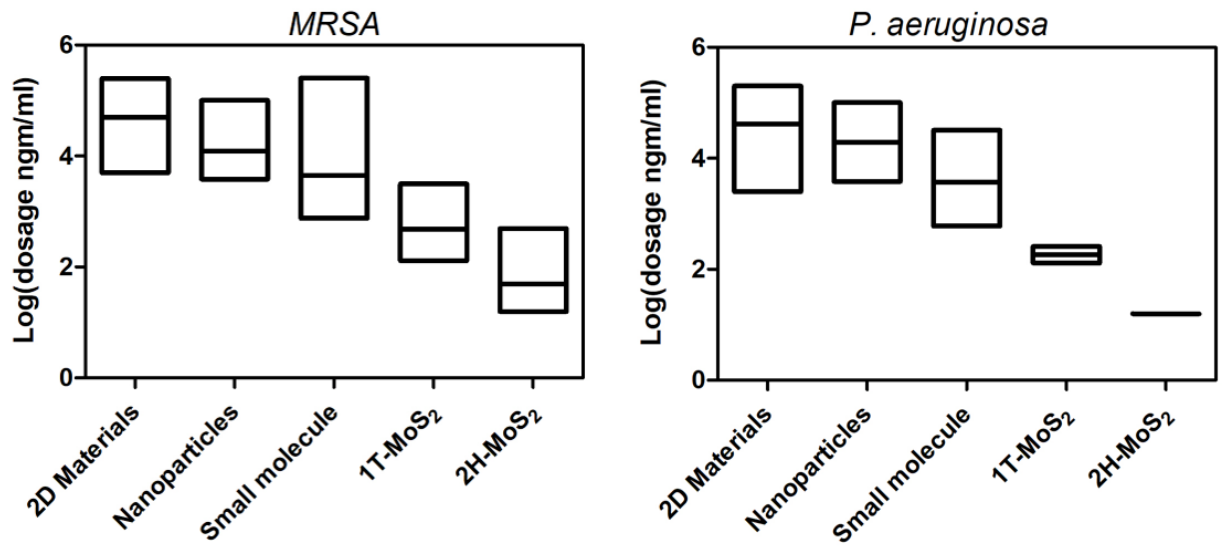

Figure S20. Dosage comparison of functionalized 2H-MoS_2 with existing functionalized 1T-MoS_2, 2D-nanomaterials, nanoparticles and small molecules. The dosage values of all materials adapted from previous report.[1]

22. Quenching assay to determine the type of ROS Species in 1T-MoS_2.

The quenching assay also carried in the presence of 3 ppm 1T MoS_2 (Prepared by lithium intercalation) to estimate the type of ROS species which can be generated by 1T MoS_2. Only catalase shows the reduction of percentage loss of GSH. Hence it is found that the peroxide is the only one type ROS species which is generated in 1T-MoS_2.

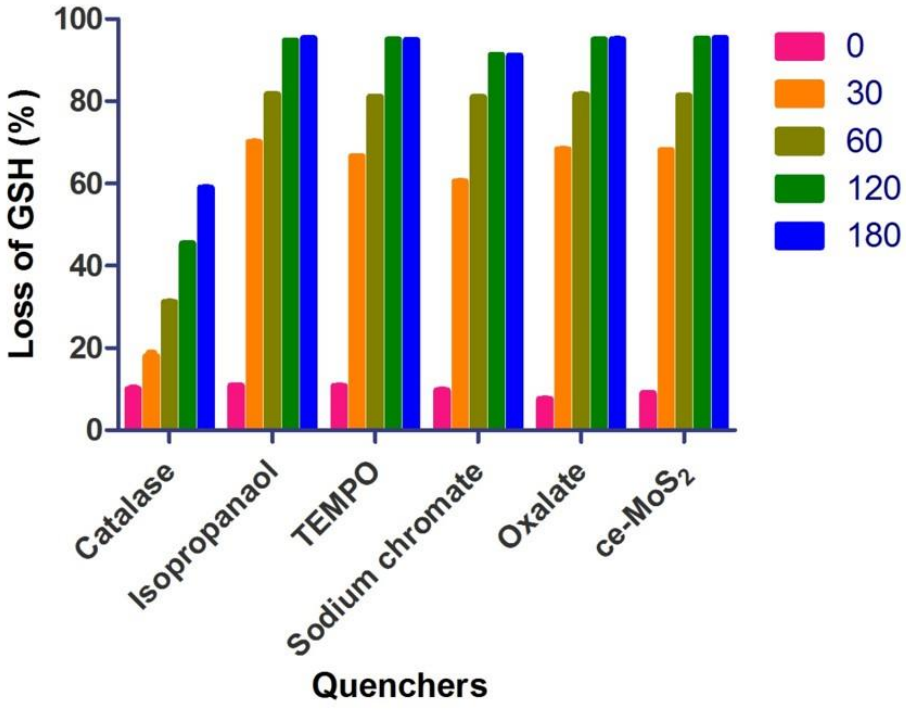

Figure S21. Ellman's assay in the presence of different quencher with 1T MoS_2 at different time interval.

23. TEM images of MRSA.

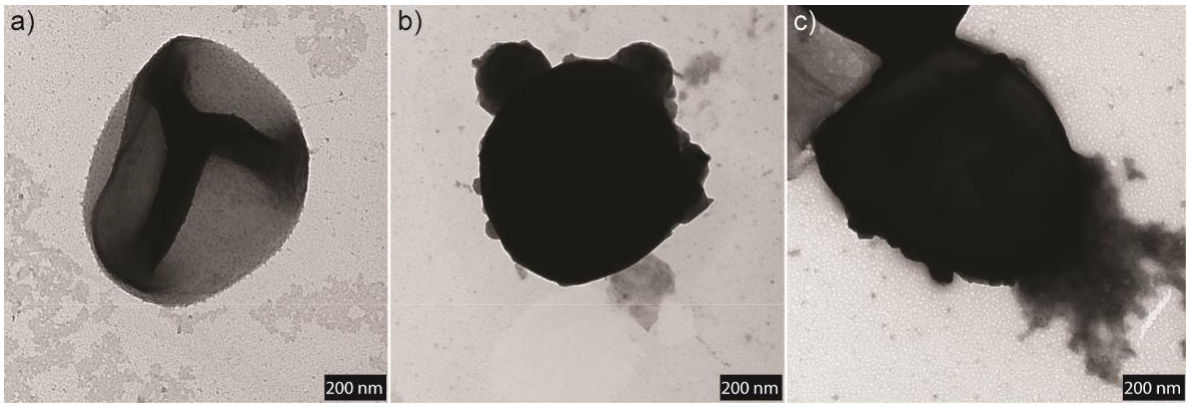

Figure S22. TEM images of MRSA a) Control without treatment. b) MRSA treated with 1xMIC of **ligand 3**-MoS_2. c) MRSA treated with 1xMIC of **ligand 8**-MoS_2. Due to the high staining of MoS_2 layers and small size of MRSA bacteria cell appears as very dark images.

24. *P. aeruginosa* treated with ligand 3-MoS_2 elemental mapping of Mo & S merged image.

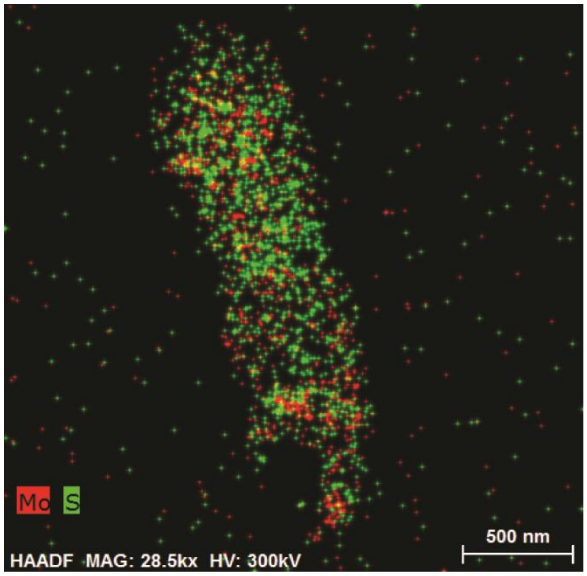

Figure S23. Merged image of elemental mapping of Mo and S for *P. aeruginosa* treated with **ligand 3**-MoS_2. The analysis indicates the binding of MoS_2 layers with bacteria cell.

25. Energy dispersive spectroscopy (EDS) spectra of *P. aeruginosa* treated with ligand 3-MoS_2.

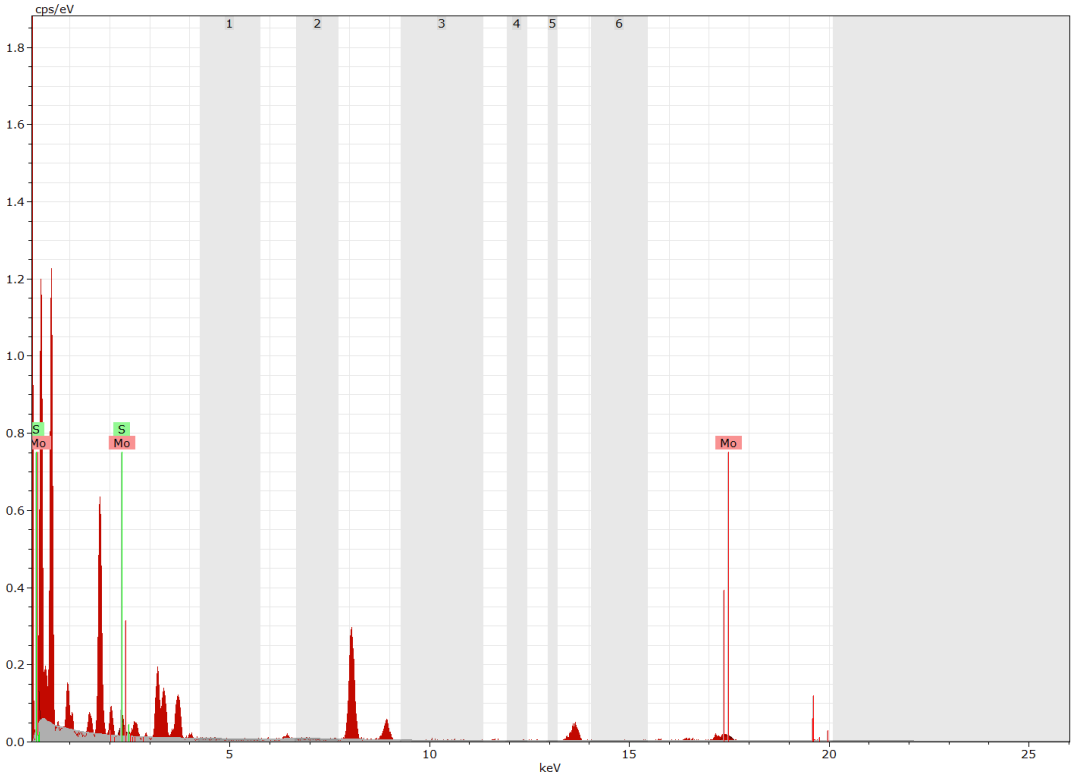

Figure S24. Energy dispersive spectra of *P. aeruginosa* treated with **ligand 3**-MoS_2. The presence of Mo indicates the binding of functionalized MoS_2 with bacteria cell wall.

26. Hemolysis assay.

Hemolysis assay performed on Rabbit RBCs. The 2 mL blood collected from Rabbit, immediately RBCs washed with PBS and centrifuged at 5000 rpm. After washing 1 mL RBCs re-dissolved in 25 mL of PBS. Then 300 µL of RBC (final RBCs conc 2%) added to a vial containing 300 µL of MoS_2 with PBS. For positive control 0.1% of Triton X-100 and PBS as a negative control, incubated at 37°C. After 1 h samples are centrifuged at 5000 rpm and taken 200 µL of supernatant and measured absorbance hemoglobin at 570 nm. The percentage of hemolysis calculated as follows

$$\%\, Hemolysis = \frac{\text{Sample absorbance} - \text{absorbance of negative control}}{\text{absorbance of postive control} - \text{absorbanceof negative control}} \times 100$$

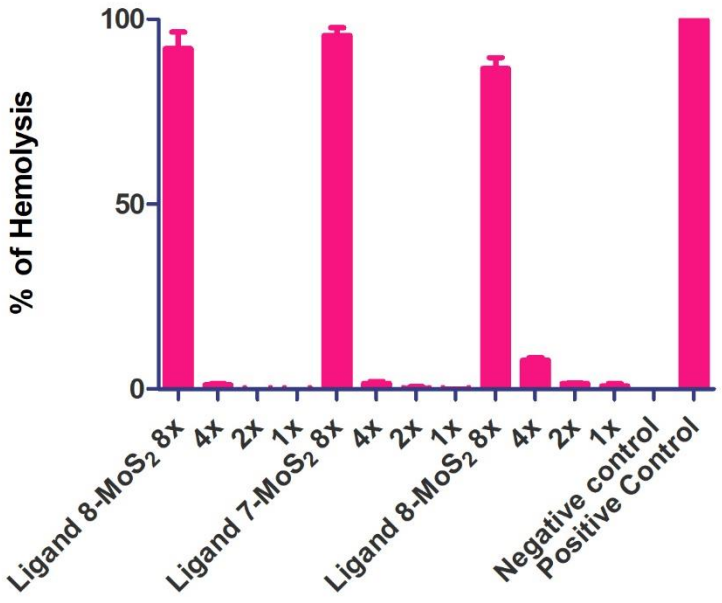

Figure S25. After 24 h, effect of functionalized MoS_2 on RBC.

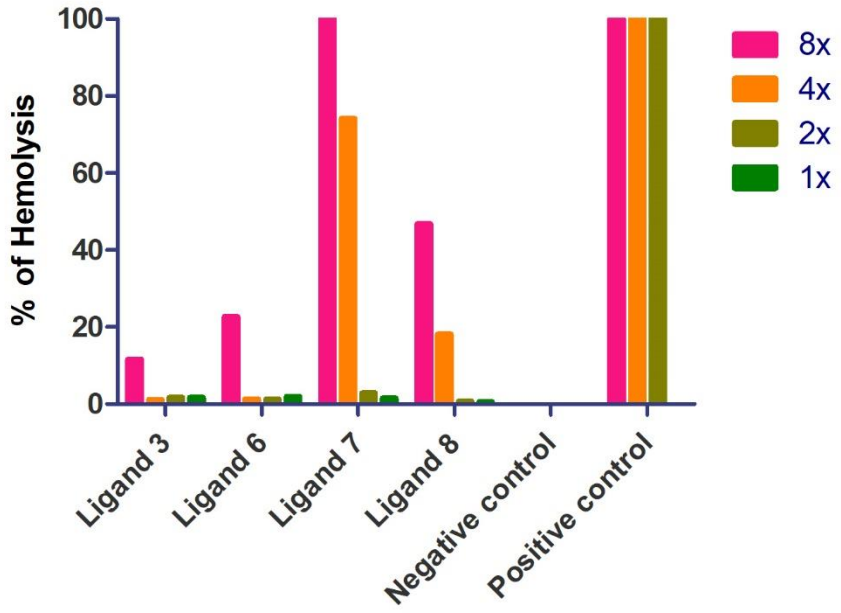

Figure S26. Effect of free ligands on RBC hemolysis after 1 h incubation.

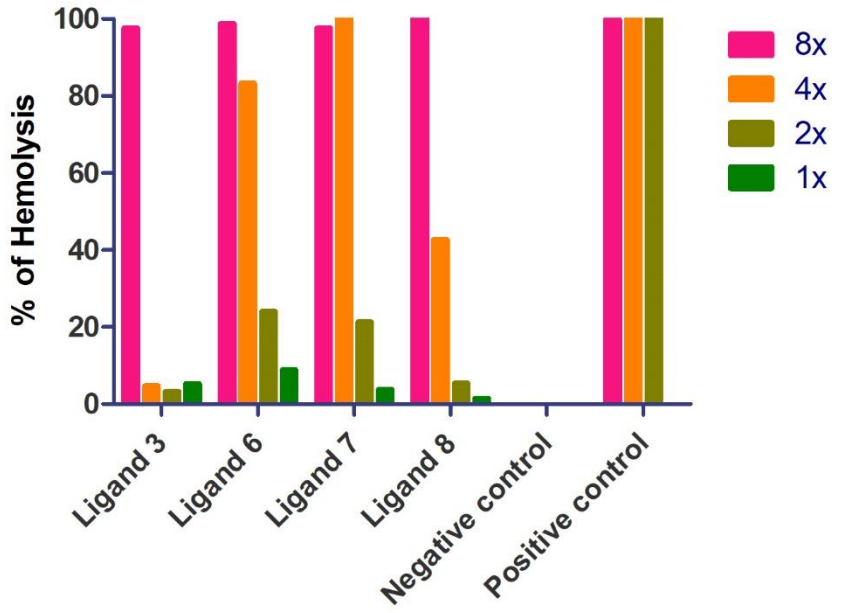

Figure S27. Effect of free ligands on RBC hemolysis after 24 h incubation.

References.

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